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A STUDY OF METHODS SUITABLE FOR THE ANALYSIS AND PRESERVATION OF NITROGEN FORMS IN AN ESTUARINE ENVIRONMENT

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U.S. PUBLIC HEALTH SERVICE

REGION IX

WATER SUPPLY AND POLLUTION CONTROL DIVISION

on

A STUDY OF METHODS SUITABLE FOR THE ANALYSIS AND PRESERVATION OF NITROGEN FORMS IN AN ESTUARINE ENVIRONMENT

By

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August 1965

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I. INTRODUCTION

The study described in this report was conducted under a purchase order to The Regents of the University of California from Region IX of the U.S. Public Health Service, Water Supply and Pollution Control Division.

II. OBJECTIVES

The objectives of the study were:

1. To determine the suitability of various suggested analytical techniques for the estimation of nitrogen forms in an estuarine environment.

2. To determine the precision and accuracy of the selected methods of analysis.

3. To determine the most suitable method of preservation of samples to be analyzed for nitrogen forms.

III. METHOD OF PROCEDURE

1. Sample Collection

Sampling points were selected in the San Francisco Bay system to give waters typical of:

a. fresh water inflow to the Bay system;

b. brackish water in the Bay system; and

c. saline water offshore from the Bay system.

The samples were collected in large plastic bottles. Samples used in method development work were stored in the refrigerator without the addition of preservative. When the methods of preservation were being investigated large samples were collected early in the morning and brought to the laboratory immediately so that an initial analysis could be performed on the same day as sampling. The large samples were then split into smaller portions, placed in plastic bottles and treated with the selected preservatives.

2. General Laboratory Procedure

The forms of nitrogen studied were as follows:

a. Ammonia,

b. Total unoxidized, **(ammonia + total organic)**

c. Soluble unoxidized, (ammonia + soluble organic)

d. Nitrite,

e. Nitrate.

Methods that appeared to be most suitable were selected by a review of the literature. These methods were then tested in the laboratory for interferences, accuracy, reproducibility, and recovery on samples from the three areas of the Bay. Following the development of a satisfactory method of analysis for each of the nitrogen forms a series of preservation experiments was conducted to determine the most suitable method of sample preservation.

IV. RESULTS OF STUDY

1. Ammonia Nitrogen (NH_3-N)

a. Method development

1) Pyrazolone method: An attempt was made to adapt the pyrazolone method of Kruse and Mellon [1] which had been applied to sea water by Strickland and Parsons [2]. In this technique the sample is treated directly with chloramine T and a "pyrazolone" reagent in the presence of pyridine. A yellow-colored complex is formed with ammonia which is then extracted into carbon tetrachloride and determined colorimetrically.

The method is subject to interference by high salt concentrations which produce a decrease in response. In a medium such as ocean water where the salt content is essentially constant Strickland and Parsons [2] found that it was possible to correct for the salt effect by running standards made up in sea water specially selected for its low phosphate content. In an estuarine situation this approach is not applicable because of the wide variation of salt concentrations encountered.

A salt masking technique similar to that suggested by Jenkins and Medsker [3] for a nitrate technique was applied to the pyrazolone method. For a given concentration of NH_3-N the optical density (OD) of the pyrazolone-ammonia complex was constant over the range of 20-40 g Cl/ℓ.

This fact was utilized by adding sodium chloride to each sample to give an added concentration of 20 g Cl/ℓ for the purpose of masking out the effect of varying salt concentrations in samples containing from 0 to 20 g Cl/ℓ.

Even when the salt masking technique was utilized the pyrazolone method proved to be unacceptable for the direct analysis of saline water samples although it performed satisfactorily (without salt masking) on distilled samples. The following experiences with the method are given as a

basis for its rejection in favor of the Nesslerization of a distilled sample:

i) Two batches of pyrazolone reagent made up from the same chemicals may give two quite different, non-linear standard curves.

ii) The "pyrazolone" reagent begins to decompose in less than two hours.

iii) The per cent recovery of added NH_3-N is not constant and varies with the nature of the water being analyzed and with its NH_3-N content.

iv) The procedure is time-consuming and involves the use of several reagents which are susceptible to contamination by impurities in the chemicals used to prepare them.

2) Distillation and Nesslerization

Nesslerization of a distilled sample is the "classical" method of NH_3-N determination and has been shown in this laboratory to be reliable and accurate if performed carefully and in accordance with the procedure outlined in the appendix of this report.

The samples are distilled in a weakly basic solution buffered with sodium carbonate [4]. The sodium carbonate buffer is preferred over the phosphate buffer system recommended by Standard Methods [5] because the latter must be used in large quantities (due to its precipitation by the Ca^{+2} and Mg^{+2} present in saline samples) and causes a high blank value in the test. The distillate is trapped in a weak solution of boric acid and Nesslerization is performed on this distillate.

Since the possibility of decomposing organic nitrogen compounds during distillation has frequently been suggested, an attempt was made to check whether this was occurring in the samples from the Bay. No direct method of checking this effect is available but the following "circumstantial" evidence strongly indicates that there is no significant release of ammonia from organic nitrogen compounds during the distillation procedure.

i) Three Bay water samples obtained at different locations in the Bay (Pittsburg, Crockett, and near the Golden Gate) were analyzed for NH_3-N in both the whole and Millipore (HA) filtered portions. The NH_3-N content of both aliquots of the three samples was the same.

It can thus be concluded that suspended solids did not contain any decomposable organic nitrogen nor was any NH_3-N being extracted from the suspended solids.

ii) Since a considerable quantity of soluble organic nitrogen was present in the samples its behavior during distillation was investigated.

Water obtained from Crockett which had a soluble organic nitrogen content of about 415 $\mu g/\ell$ was filtered through an HA Millipore filter and NH_3-N analyses were performed on the filtrate using several different times of distillation or, in essence, collecting several different volumes of distillate. The NH_3-N recovered reached a constant value when between 150-300 $m\ell$ of distillate had been collected from an original volume of 350 $m\ell$. Below this range recovery was incomplete and when more than 300 $m\ell$ was distilled there was an indication that decomposition had started (Figure 1).

iii) Although the pyrazolone extraction method was rejected as unreliable for undistilled saline water samples, the NH_3-N values obtained using this method agree semi-quantitatively with those obtained by Nesslerization of the distillates.

Because of the massive quantity of cations other than NH_3-N present in saline samples it was found to be impossible to remove NH_3-N by ion exchange and study the decomposition of organic nitrogen alone. Moreover, it is conceivable that nitrogen-containing organic substances may be removed by an ion-exchange technique which would further invalidate this approach.

Interferences with the Nesslerization procedure can be largely eliminated by the distillation step. If volatile organic compounds are present, they will readily distill over together with the NH_3-N. Previous experience with the Bay water samples had indicated that negligible interferences were caused in this manner. A far more important source of trouble in the Nesslerization procedure is the accidental contamination of solutions or glassware.

The Nesslerized samples frequently respond to contamination by developing turbidity as well as becoming more highly colored. Extreme caution must be exercised to avoid introducing contaminants when working in the low microgram range. Opening a bottle of ammonia or smoking in the vicinity where these analyses are being carried out must be prohibited. Glassware must be kept meticulously clean and distilled water produced by ion exchange must be used throughout the procedure as well as in the preparation of reagents.

b. Recovery and replication

The technique given in Appendix I was tested for recovery of NH_3-N from Bay water samples of widely varying salinity. The

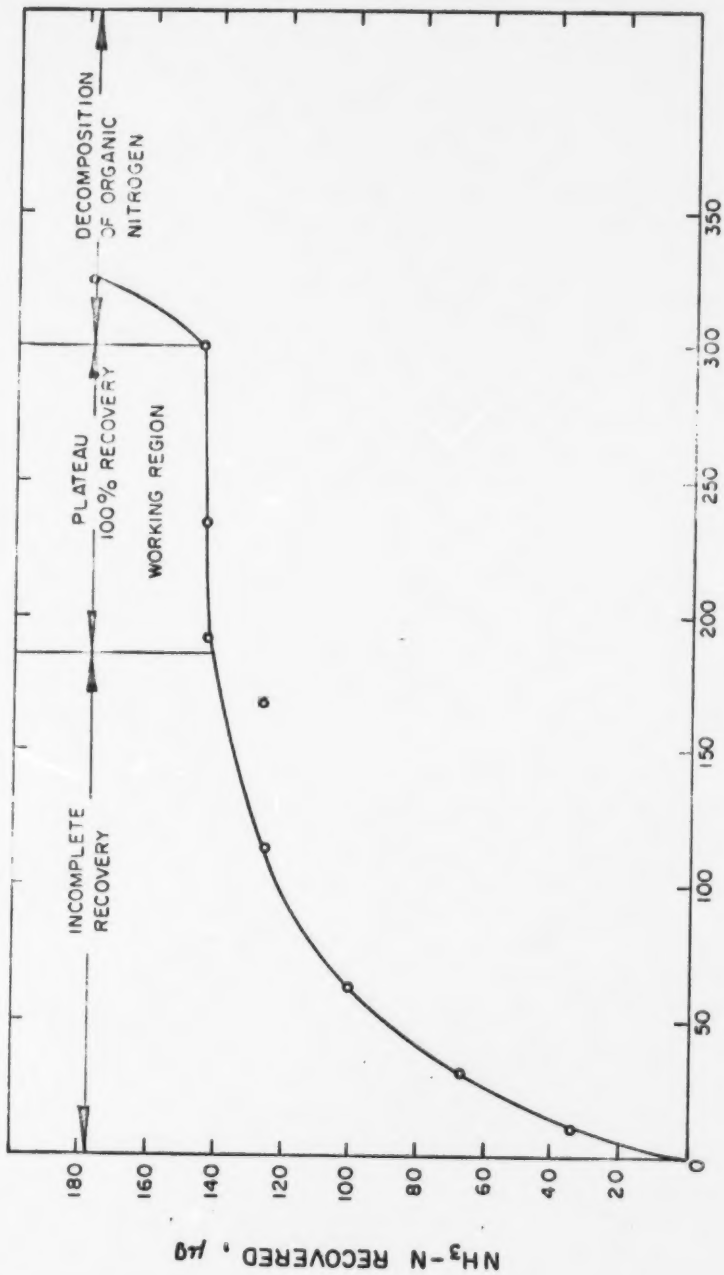

FIGURE 1. RECOVERY OF NH_3-N WITH DIFFERENT VOLUMES OF DISTILLATE

results, presented in Table 1, show that excellent recovery of 100 μg NH_3-N/ℓ is possible using the technique.

The precision of the technique was investigated by the analysis of 10 replicates of samples from three regions of the Bay. At a level of about 110 μg NH_3-N/ℓ the coefficient of variation (C_v) was 1.8 per cent; at a level of about 65 μg NH_3-N/ℓ the C_v was 6.9 per cent while at about 35 μg NH_3-N/ℓ the C_v increased to about 13 per cent. It would seem that the method loses its excellent precision below NH_3-N concentrations of about 60 μg/ℓ. (Table 2 and Figure 2).

2. Total Unoxidized Nitrogen **(Total Kjeldahl Nitrogen)**

a. Method development

The classical digestion method first proposed by Kjeldahl [6] was used in the present work and adopted as a satisfactory means of determining the total quantity of trinegative nitrogen in Bay waters. Concentrated sulfuric acid is added both as an oxidizing agent and to fix the ammonia as ammonium sulfate; potassium sulfate is used to raise the boiling point of the acid to between 345°C and 370°C. To avoid raising the boiling point over 380°C, which results in a loss of ammonia, it is recommended by Swift [7] that an H_2SO_4/K_2SO_4 molar ratio of greater than 1.5 be maintained. After considering the ionic contributions from samples ranging from river water to sea water as well as the expected loss of H_2SO_4 during digestion, the quantities of acid and salt given in the Appendix were adopted as adequate to satisfy the ratio proposed by Swift [7].

For convenience, Kelpak, a commercially available packaged product, was used to introduce the K_2SO_4. Each package of Kelpak also contained a small quantity of copper sulfate catalyst and a little pumice to promote smooth boiling during digestion.

Although copper salts are generally recognized as slightly less effective as catalysts than mercuric salts, their use shortens the analytical procedure and has yielded results in Bay water samples that were comparable with those obtained using a mercury catalyst. After digestion, sodium hydroxide is added to neutralize the acid and to raise the pH into the alkaline range which is necessary for distillation of the ammonia. The procedure then follows that used for the ammonia determination using Nesslerization to estimate the ammonia in the distillate.

b. Recovery and replication

The recovery of 50 μg of ammonia nitrogen by the total unoxidized nitrogen technique was tested on Millipore-filtered Bay water samples of widely differing salinity. Excellent results were obtained (Table 3). The efficacy of the digestion procedure in releasing nitrogen from organic material was

TABLE 1

RECOVERY OF ADDED NH_3-N FROM SAN FRANCISCO BAY WATER

Location and Date of Sampling	Suspended Solids mg/ℓ	Chloride mg/ℓ	NH_3-N µg/ℓ	NH_3-N Added µg	NH_3-N Recovered µg	Recovery %
PITTSBURG 5/17/65 Low Tide	38	120	62.5	100	96	96
CROCKETT 5/17/65 Low Tide	194	1010	116	100	95	95
GOLDEN GATE 5/17/65 Low Tide	10	14400	31.5	100	95	95

TABLE 2

REPLICATE NH_3-N ANALYSES OF THREE SAN FRANCISCO BAY WATER SAMPLES

NH_3-N, µg/ℓ		
GOLDEN GATE 5/17/65 Low Tide	PITTSBURG 5/17/65 Low Tide	CROCKETT 5/17/65 Low Tide
30.1	65.0	114
34.8	65.0	111
42.8	60.3	110
34.8	73.0	110
32.4	69.5	114
30.1	67.2	109
41.7	71.8	109
39.4	60.3	114
34.8	61.5	110
34.8	65.0	111
Mean		
35.57	65.86	111.2
Standard Deviation		
4.69	4.53	2.04
Coefficient of Variation, %		
13.2	6.9	1.8

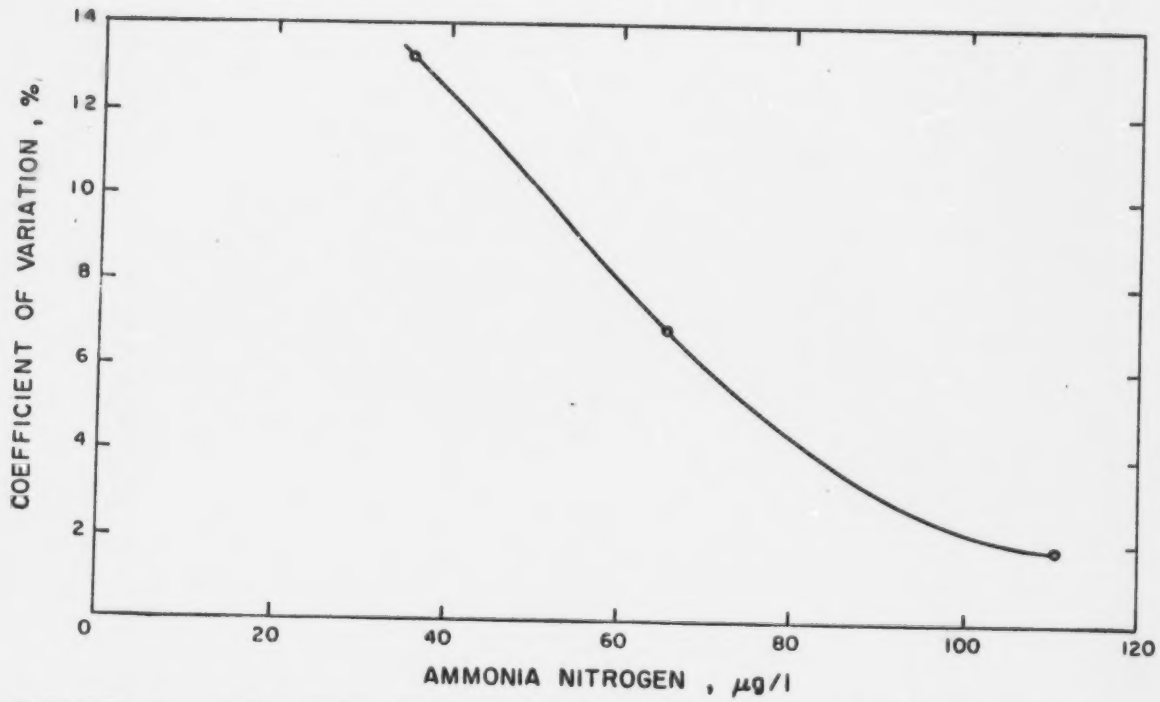

FIGURE 2. PRECISION OF NH_3–N TECHNIQUE AT VARIOUS LEVELS OF NH_3 –N

TABLE 3

RECOVERY OF ADDED NH_3-N AND ORGANIC N FROM SAN FRANCISCO BAY WATER BY THE TOTAL UNOXIDIZED NITROGEN TECHNIQUE

Location and Date of Sampling	Chloride mg/ℓ	Total Unoxidized N µg/ℓ	NH_3-N Added µg	NH_3-N Recovered µg	Recovery %
PITTSBURG 5/17/65 Low Tide	120	330	50	50.3	101
CROCKETT 5/17/65 Low Tide	1010	480	50	48.8	98
GOLDEN GATE 5/17/65 Low Tide	14400	150	50	47.7	95
106.6 mg Dry Algae, % Nitrogen					
--	--	6.2	--	--	6.4
590 µg Casein, % Nitrogen					
--	--	15.8	--	--	14.9

checked by digesting a sample of algae whose nitrogen content had been determined by elemental analysis [8]. A content of 6.4 per cent N was obtained by the recommended procedure while 6.2 per cent N was found by elemental analysis. As a further check the nitrogen content of the protein casein was determined; a nitrogen content of 14.9 per cent was obtained while 15.8 per cent N is listed by the Merck Index [9] as a "typical value" for the nitrogen content of casein. It would seem in the light of these results that the digestion procedure is adequate.

Replication of total nitrogen results for unfiltered Bay water was generally quite poor (Table 4). Usually, out of ten replicates of a sample analyzed, eight or nine were extremely close while there were one or two unusually high values. All efforts to eliminate these high values failed. Even mixing the sample for several minutes in a high-speed Waring blender had no effect on the situation. Coupled with the excellent results for replication shown by the use of the total unoxidized nitrogen technique for filtered Bay water samples, it would seem that the poor reproducibility of the total unoxidized nitrogen test on unfiltered samples is due to the inhomogeneity of the samples caused by uneven distribution of suspended material.

TABLE 4

REPLICATE TOTAL UNOXIDIZED NITROGEN ANALYSES ON THREE UNFILTERED AND ONE MILLIPORE-FILTERED SAN FRANCISCO BAY WATER SAMPLES[a]

Total Unoxidized Nitrogen, µg/ℓ			
PITTSBURG Unfiltered	PITTSBURG Filtered	CROCKETT Unfiltered	GOLDEN GATE Unfiltered
373	328	675	233
377	348	688	233
389	316	972	252
370	353	673	396
382	348	658	254
393	333	658	241
398	333	652	262
379	351	657	274
377	339	660	236
387	351	726	268
Mean			
382	340	702	265
Standard Deviation			
9.02	12.3	97.3	48.3
Coefficient of Variation, %			
2.36	3.61	13.9	18.2

[a]Samples taken 5/17/65 on a Low Tide.

3. Soluble Unoxidized Nitrogen

a. Method development

The Kjeldahl method, as described for total unoxidized nitrogen, was used. The samples were filtered through washed HA Millipore filters and the analysis performed on the filtrate. It is important to wash the HA Millipore filters before using them to separate the suspended and soluble forms of nitrogen because it was found that the filters contain some soluble material that contributes significantly to both the ammonia and total nitrogen content of the filtrate. The washing of filters can be performed by soaking them in deionized distilled water

for two hours, replacing the water and then soaking the filters overnight.

b. Replication

Since suspended matter, which was the suspected source of variation encountered in the total unoxidized nitrogen technique, was removed by filtration it was possible to obtain good replication of the soluble unoxidized nitrogen technique (Table 4). For ten replicates of a sample with a mean soluble unoxidized nitrogen content of 340 µg/ℓ a coefficient of variation of 3.6 per cent was obtained.

4. Nitrite Nitrogen

a. Method development

Diazotization and coupling reactions involving the nitrite ion may be carried out with many combinations of electrophilic reagents (Kodama [10]). In the method adopted for this study NO_2-N is reacted in the sulfanilic acid to form a diazonium ion which is then coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a purple-colored azo dye whose color can be measured spectrophotometrically.

The presence of suspended matter in the water samples was found to decrease the recovery of added NO_2-N. It was postulated that the suspended solids adsorbed the azo dye from solution since the recovery decreased with increasing suspended solids concentrations (Table 5). It is therefore recommended that samples for NO_2-N analysis be filtered through HA Millipore filters before analysis.

TABLE 5

EFFECT OF SUSPENDED SOLIDS ON THE RECOVERY OF NO_2-N FROM BAY WATER SAMPLES

Suspended Solids mg/ℓ	Recovery of NO_2-N %
12	86
20	53
34	47

b. Recovery and replication

Recovery of 1.0 and 2.0 µg of added NO_2-N from Bay water samples covering the salinity range commonly encountered in San Francisco Bay was excellent and averaged 98 per cent (Table 6). Replication of the method in waters with a

TABLE 6

RECOVERY OF ADDED NO_2-N FROM THREE SAN FRANCISCO BAY WATER SAMPLES

Location and Time of Sampling	Suspended Solids mg/ℓ	Chloride mg/ℓ	NO_2-N µg/ℓ	NO_2-N Added µg	NO_2-N Recovered µg	Recovery %
ANTIOCH BRIDGE 4/27/65 12:45 P.M.	34	150	3.0	1.00 2.00	1.01 1.90	101 95
CROCKETT 4/27/65 11:30 A.M.	20	3850	5.6	1.00 2.00	0.99 1.90	99 95
SAUSALITO 4/27/65 9:15 A.M.	12	12300	8.4	1.00 2.00	0.94 2.04	94 102

chlorosity of 150, 3850, and 12300 mg/ℓ and containing a mean NO_2-N content of 2.21, 5.60, and 7.96 µg NO_2-N/ℓ, respectively was excellent, showing a coefficient of variation never greater than 2.6 per cent (Table 7).

5. Nitrate Nitrogen

a. Method development

The nitrate nitrogen technique of Jenkins and Medsker [3] was adopted with the exception that it is now recommended that highly turbid samples be filtered through an HA Millipore filter prior to NO_3-N analysis.

b. Recovery and replication

The following values for recovery and precision are quoted from Jenkins and Medsker [3] whose work was conducted on San Francisco Bay water.

The recovery of 0.2 mg/ℓ added NO_3-N was excellent (Table 8) using three different lots of brucine sulfate purchased from different manufacturers.

The coefficient of variation of the test was about 1.7 per cent at a level of about 0.29 mg NO_3-N/ℓ (Table 9).

TABLE 7

REPLICATE NO_2-N ANALYSES ON THREE SAN FRANCISCO BAY WATER SAMPLES

Nitrite Nitrogen, µg/ℓ		
ANTIOCH BRIDGE 4/27/65, 12:45 P.M.	CROCKETT 4/27/65, 11:30 A.M.	SAUSALITO 4/27/65, 9:15 A.M.
2.26	5.56	7.76
2.22	5.68	7.94
2.14	5.72	8.06
2.14	5.80	7.88
2.32	5.48	7.88
2.08	5.56	8.06
2.26	5.48	8.18
2.26	5.48	7.94
2.32	5.80	8.18
2.14	5.80	7.76
Mean		
2.214	5.596	7.964
Standard Deviation		
0.0266	0.145	0.152
Coefficient of Variation, %		
1.20	2.56	1.92

TABLE 8

RECOVERY OF NO_3-N FROM VARIOUS SAMPLES USING THE MODIFIED BRUCINE TECHNIQUE WITH THREE DIFFERENT LOTS OF BRUCINE

Type of Water Sample		Brucine Lot No.	NO_3-N, mg/ℓ			
Source	Chloride g/ℓ		Present Initially	NO_3-N Added	NO_3-N Recovered	Recovery %
San Pablo Bay	14.3	I	0.18	0.20	0.38	100
		II	0.19	0.20	0.39	100
		III	0.19	0.20	0.40	105
North San Francisco Bay	15.8	I	0.13	0.20	0.33	100
		II	0.14	0.20	0.33	95
		III	0.14	0.20	0.35	105
Sacramento River	0.010	I	0.07	0.20	0.26	95
		II	0.07	0.20	0.26	95
		III	0.08	0.20	0.28	100

TABLE 9

PRECISION OF MODIFIED BRUCINE TECHNIQUE FOR NO_3-N DETERMINATION

NO_3-N, mg/ℓ
0.28 0.29 0.29 0.29 0.28 0.29 0.29 0.28 0.29 0.29
Mean
0.287
Standard Deviation
0.0048
Coefficient of Variation, %
1.7

V. PRESERVATION STUDY

1. Method of Procedure

a. Sampling methods

Early in the morning of 28 June 1965 water samples from three widely separated points in the San Francisco Bay (Antioch Boat Ramp, Rodeo Marina, and Fort Baker) were collected in three separate 5-gal plastic bottles. The samples arrived at the laboratory at about 0900 at which time their temperature was between 20-22°C.

b. Preservation

Each large 5-gal sample was thoroughly mixed by swirling and then split into 28 portions of about 500 mℓ each which were introduced into small, wide-mouth plastic bottles. The 28 small portions were further divided into 4 groups of 7 each. These subgroups of 7 samples were then treated as follows:

i) Stored at 4°C; no preservative added.

ii) Stored in a deep freeze at −10°C; no preservative added.

iii) Stored at 4°C; 5% sulfuric acid added at a rate of 2 mℓ/ℓ.

iv) Stored at 4°C; 40 mg/ℓ Hg^{++} added (as $HgCl_2$).

Immediately upon receiving the samples in the laboratory a complete set of analyses was performed to establish the initial concentrations of the various forms of nitrogen. For the sample preserved with 5% H_2SO_4 a complete set of analyses was also performed immediately after acid-treating the samples to determine if the addition of acid had any immediate effect on the concentration of the various forms of nitrogen.

Changes in the various forms of nitrogen were observed in the samples at various time intervals for a period of 42 days. A separate bottle was removed each time a series of analyses was performed so that no disturbance of the quiescent preservation conditions was caused by the mixing that would be required to withdraw an aliquot from a large sample.

c. Analyses

The analyses for the various forms of nitrogen were carried out in accordance with the methodology given in Appendix A.

2. Results of Preservation Study

a. General

Several difficulties, associated with the amount of analytical work and the large volume of samples that had to be collected, were encountered in the preservation study. It was not possible to carry out replicate analyses for each form of nitrogen on specific days in each sample. There are two reasons for this: firstly, the volume of analytical work which had to be performed during each day of analysis was already too much for one person when only a single analysis for each form of nitrogen was performed on all of the three samples and the four methods of preservation applied to each of the samples. Secondly, to set up the replicate samples or to provide sufficient volume so that replicates could be carried out on each sample would have required the collection of a much larger volume of water than was collected. While this may have been practical, it would have been impractical to thoroughly mix a larger

volume so that sub-samples withdrawn from the large volume would be representative. For these reasons replication during this preservation study was not practical.

b. Results

So that conclusions will not be drawn from behavior of single sub-samples the results of the same preservation method for the three samples from the widely different areas of the Bay have been averaged and the changes in each nitrogen form expressed as a percentage change.

The location and time of sampling, suspended solids content, chlorosity, and initial concentration of nitrogen forms of the three samples is summarized in Table 10. The results of the study are discussed for each form of nitrogen analyzed and the changes in the different forms of nitrogen on an average percentage basis are illustrated in Figure 3 through 6 and summarized in Table 11 through 14.

1) Nitrate Nitrogen

It should be noted that in the majority of the samples tested the NO_3-N concentration was below 0.1 mg/ℓ and in all cases below 0.15 mg/ℓ. The accuracy of the method, determined in a prior study by Jenkins and Medsker [3], was made on a sample containing about 290 µg NO_3-N/ℓ. It might be expected that the error would be greater in this study since the levels of nitrate were much lower and the analytical work more hurried. In view of these factors it is difficult to place a true estimate of significance on the changes observed in NO_3-N during storage.

a) Stored at 4°C; no preservative.

Over the entire storage period NO_3-N concentrations were higher than initial values and after 42 days storage averaged 44 per cent higher than the initial values.

b) Stored at -10°C; no preservative.

There was no consistent variation of NO_3-N concentration over the 42 days of storage.

c) Stored at 4°C; acid preservative.

The average NO_3-N was slightly higher than the initial value for the whole of the storage period but did not show any significant upward trend during the 42-days of storage.

TABLE 10

SAMPLING AND ANALYTICAL DATA FOR SAMPLES USED IN THE PRESERVATION STUDY

Location	Time of Sampling	Sample No.	SS (mg/ℓ)	Cl (mg/ℓ)	Nitrogen Form, μg N/ℓ				
					NO_3	NO_2	NH_3	Soluble Organic	Insoluble Organic
Antioch Boat Ramp	0645	1	68	10	70	2.98	34.7	410	113
Rodeo Marina	0815	2	308	9,260	51	6.67	436	364	760
Fort Baker	0700	3	20	13,200	115	6.72	141	200	46

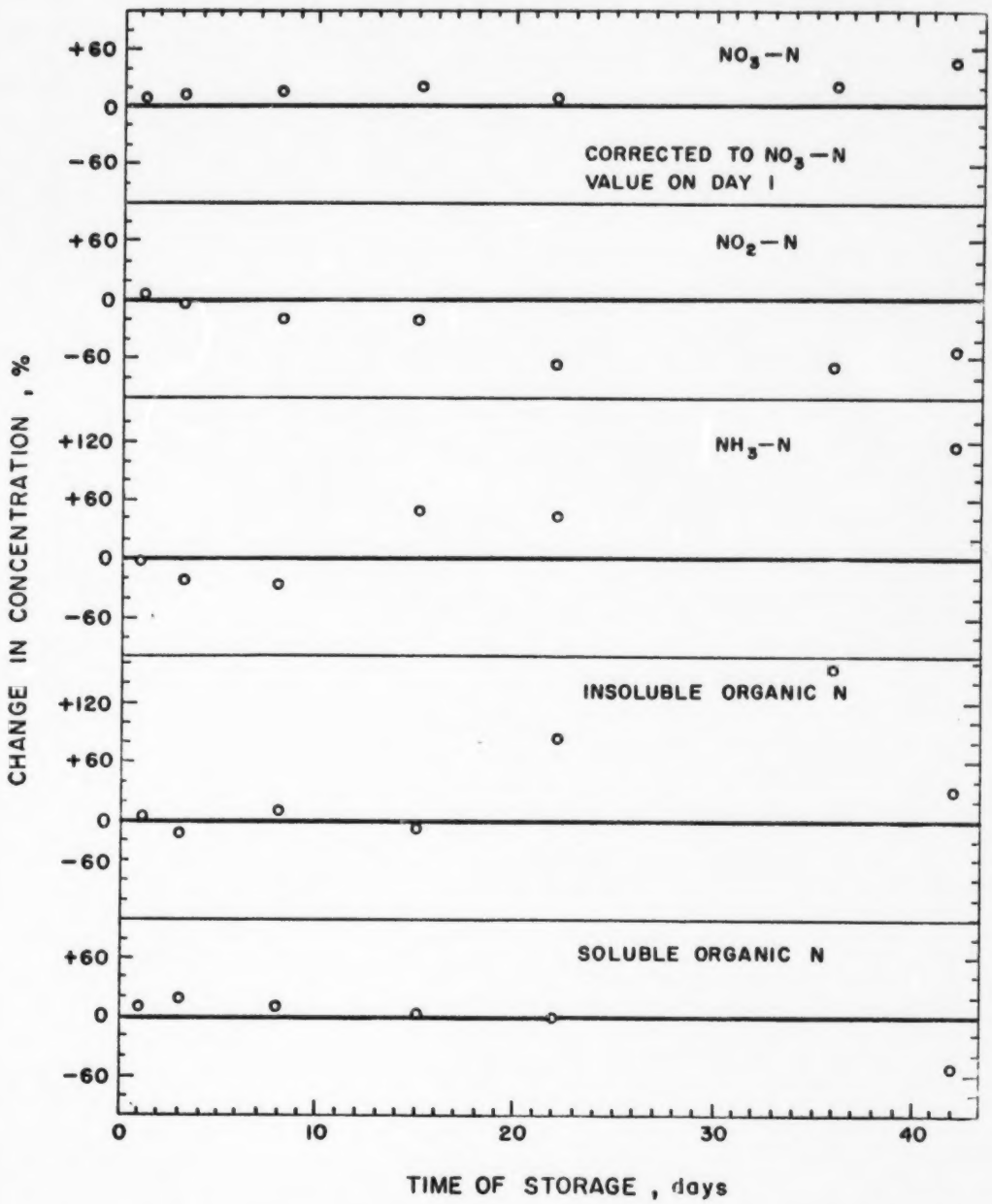

FIGURE 3. PER CENT CHANGE IN CONCENTRATION OF NITROGEN FORMS DURING STORAGE AT 4°C WITH NO PRESERVATIVE

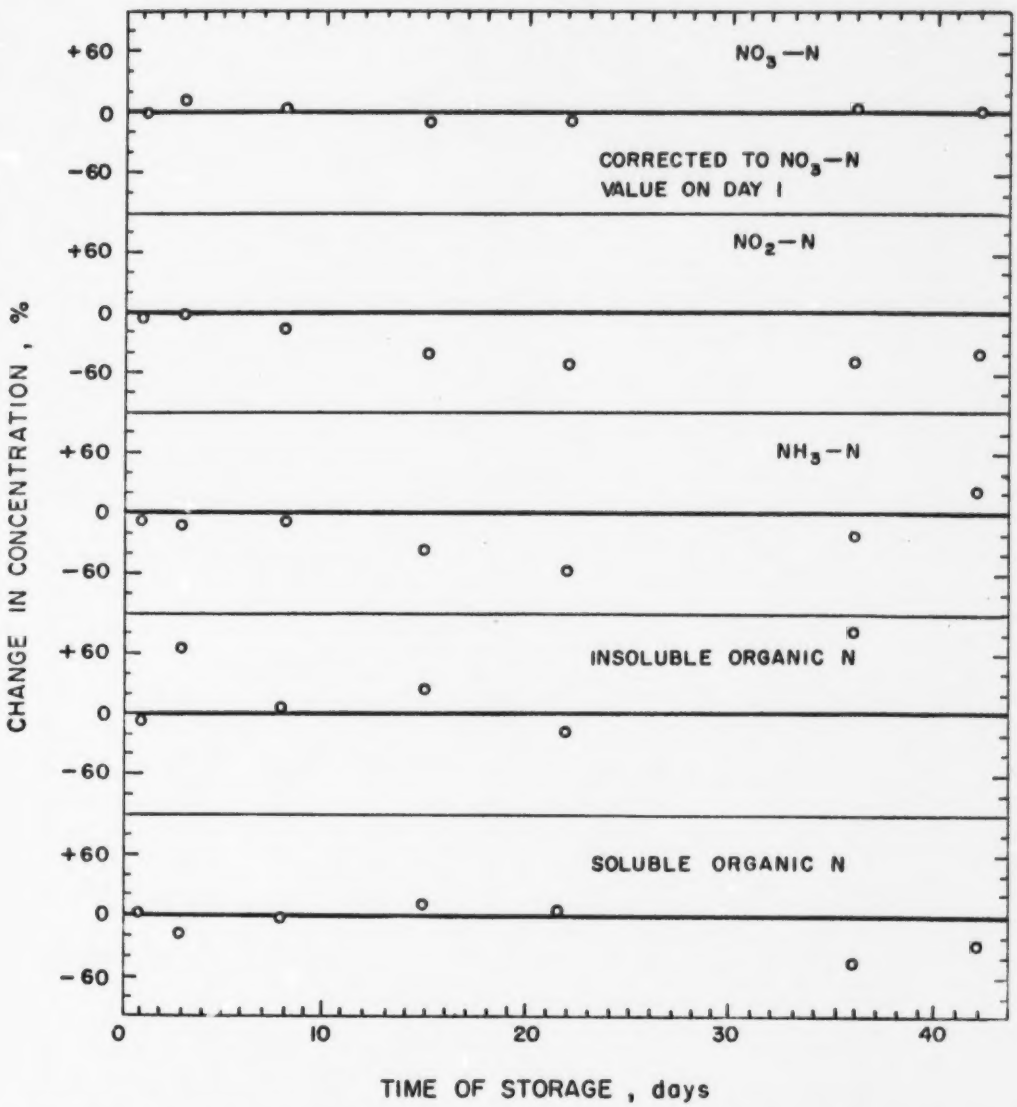

FIGURE 4. PERCENT CHANGE IN CONCENTRATION OF NITROGEN FORMS DURING STORAGE IN A FROZEN STATE AT -10°C

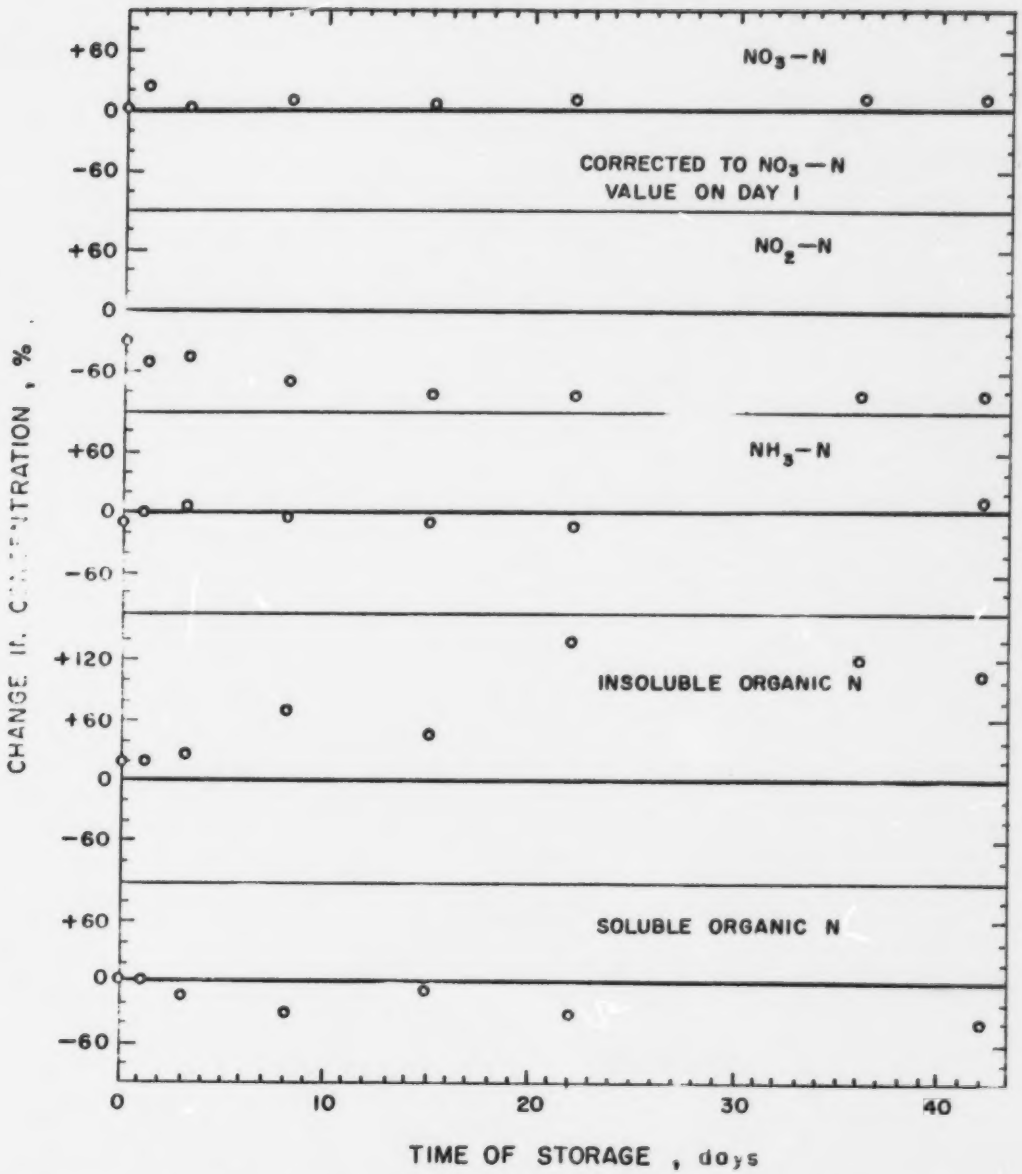

FIGURE 5 . PER CENT CHANGE IN CONCENTRATION OF NITROGEN FORMS AT 4°C WITH 2 ml 5% H_2SO_4/l ADDED AS A PRESERVATIVE

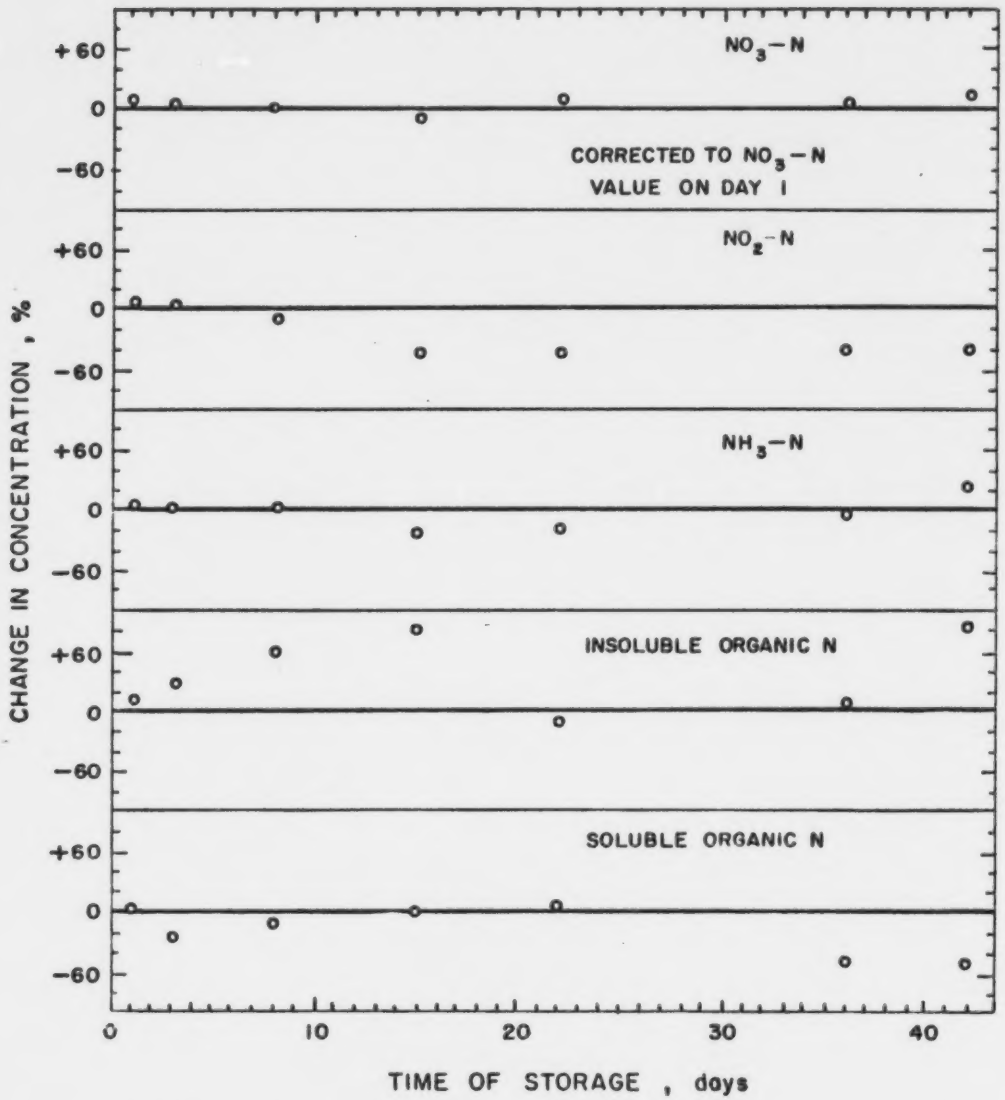

FIGURE 6. PER CENT CHANGE IN CONCENTRATION OF NITROGEN FORMS DURING STORAGE AT 4°C IN THE PRESENCE OF 40 mg/l Hg^{++} AS A PRESERVATIVE

TABLE 11

CHANGES IN CONCENTRATION (ACTUAL AND PER CENT[a]) OF NITROGEN FORMS AT 4°C WITH NO PRESERVATIVE

Nitrogen Form	Sample No.[b]	Time of Storage, days														
		0	1		3		8		15		22		36		42	
		µg/ℓ	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%
NO_3	1	70	75	+7	75	+ 7	72	+ 3	77	+ 10	62	-11	67	- 4	90	+28
	2	~ 0	52	--	57	+10[c]	67	+29[c]	65	+ 25	57	+10[c]	85	+64[c]	98	+88[c]
	3	115	130	+13	127	+10	127	+10	132	+ 15	132	+15	117	+ 2	132	+15
	Mean			+10		+ 9		+14		+ 17		+ 5		+21		+44
NO_2	1	2.98	2.62	-12	2.62	-12	1.07	-64	0.81	- 73	0.66	-78	0.37	-88	0.58	-81
	2	6.67	7.37	+10	6.86	+ 3	7.05	+ 6	9.40	+ 41	1.52	-77	1.29	-81	--	--
	3	6.72	8.14	+21	6.68	- 1	5.90	-12	4.21	- 37	3.86	-43	3.49	-48	4.54	-32
	Mean			+ 6		- 3		-23		- 23		-66		-72		-57
NH_3	1	34.7	30.2	-13	11.6	-67	20.3	-42	85.0	+145	86.8	+147	--	--	145	+318
	2	436	437	0	399	- 8	339	-22	422	- 3	397	- 9	--	--	472	+ 8
	3	141	149	+ 6	136	- 4	119	-16	139	- 2	117	-17	--	--	171	+21
	Mean			- 2		-26		-27		+ 47		+40		--		+116
Insoluble Organic	1	113	103	- 9	168	+49	252	+128	118	+ 4	134	+19	157	+39	124	+10
	2	760	752	- 1	543	-28	776	+ 2	661	-13	774	+ 2	839	+10	712	- 6
	3	46	60	+23	18	-61	3	-93	33	-28	154	+235	245	+435	81	+76
	Mean			+ 4		-13		+11		-13		+85		+161		+27
Soluble Organic	1	410	437	+ 7	332	-19	223	-46	294	-28	277	-32	--	--	171	-58
	2	364	381	+ 5	578	+59	397	+ 9	448	+23	459	+26	--	--	234	-36
	3	200	233	+16	222	+11	214	+ 7	234	+17	210	+ 5	--	--	87	-56
	Mean			+ 9		+17		-10		+ 4		0	--	--		-50

[a]Calculated by taking the difference of the two values in µg/ℓ and dividing by the initial value.

[b]Sample Nos. 1, 2, and 3 represent samples from Antioch, Rodeo, and Fort Baker, respectively.

[c]Percentage change calculated on the basis of the second day's NO_3-N concentration (see text).

TABLE 12

CHANGES IN CONCENTRATION (ACTUAL AND PER CENT[a]) OF NITROGEN FORMS IN FROZEN SAMPLES AT -10°C WITH NO PRESERVATIVE

Nitrogen Form	Sample No.[b]	Time of Storage, days														
		0	1		3		8		15		22		36		42	
		µg/ℓ	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%
NO_3	1	70	80	+14	82	+17	80	+14	95	+36	82	+17	67	- 4	80	+14
	2	~ 0	55	-	55	0[c]	50	- 9[c]	12	-78[c]	~20	-44[c]	-	-	40	-27[c]
	3	115	100	-13	127	+10	115	0	125	+ 9	125	+ 9	130	+13	130	+13
	Mean			0		+ 9		+ 2		-11		- 8		+ 5		0
NO_2	1	2.98	2.76	- 7	2.98	0	2.27	-24	2.10	-30	1.62	-46	1.73	-42	1.53	-49
	2	6.67	7.48	+12	5.84	-12	6.15	- 8	3.26	-51	2.27	-67	3.22	-52	4.21	-37
	3	6.72	5.72	-15	6.86	- 2	5.37	-20	4.14	-38	3.93	-42	3.08	-54	4.27	-36
	Mean			- 3		- 3		-17		-40		-52		-49		-41
NH_3	1	34.7	24.6	-29	17.3	-50	30.4	-12	11.6	-67	4.35	-87	21.7	-37	53.5	+54
	2	436	448	+ 3	421	- 3	425	- 3	373	-14	305	-30	346	-21	443	+ 2
	3	141	145	+ 3	150	+ 6	-	-	-	-	-	-	123	-13	145	+ 3
	Mean			- 8		-16		- 8		-40		-58		-24		+20
Insoluble Organic	1	113	153	+35	220	+95	142	+26	210	+85	-	-	-	-	167	+47
	2	760	668	-12	665	-13	740	- 3	511	-33	624	-18	739	- 3	674	-11
	3	46	26	-43	98	+113	-	-	-	-	-	-	130	+182	90	+96
	Mean			- 7		+65		+12		+26		-18		+90		+44
Soluble Organic	1	410	414	+ 1	292	-29	358	-13	304	-26	-	-	-	-	207	-50
	2	364	364	0	354	- 3	395	+ 9	534	+47	384	+ 5	248	-32	301	-17
	3	200	196	- 2	145	-28	-	-	-	-	-	-	71	-65	159	-21
	Mean			0		-20		- 2		+11		+ 5		-48		-29

[a] Calculated by taking the difference of the two values in µg/ℓ and dividing by the initial value.

[b] Sample Nos. 1, 2, and 3 represent samples from Antioch, Rodeo, and Fort Baker, respectively.

[c] Percentage change calculated on the basis of the second day's NO_3-N concentration (see text).

TABLE 13

CHANGES IN CONCENTRATION (ACTUAL AND PER CENT[a]) OF NITROGEN FORMS AT 4 °C WITH 2 mℓ 5% H_2SO_4/ℓ ADDED AS A PRESERVATIVE

Nitrogen Form	Sample No.[b]	Time of Storage, days: 0	0[c]		1		3		8		15		22		36		42	
		µg/ℓ	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%
NO_3	1	70	70	0	97	+39	90	+29	117	+67	95	+36	95	+36	93	+33	95	+36
	2	~0	20	-	107	-	75	-30[d]	62	-42[d]	88	-23[d]	80	-25[d]	107	0[d]	108	+ 1[d]
	3	115	120	+4	138	+15	137	+19	122	+ 6	127	+ 9	137	+19	132	+15	138	+17
	Mean			+2		+27		+ 6		+10		+ 8		+10		+14		+18
NO_2	1	2.98	2.64	-11	1.64	-45	2.04	-22	0.89	-70	0.39	-87	0.66	-78	0.71	-76	0.71	-76
	2	6.67	3.44	-48	0.67	-90	0.78	-88	0.54	-92	0.49	-93	0.48	-93	0.34	-95	0.75	-89
	3	6.72	5.46	-19	4.87	-28	3.88	-32	2.88	-57	1.61	-76	1.10	-84	0.78	-88	0.78	-88
	Mean			-26		-54		-47		-73		-85		-85		-86		-84
NH_3	1	34.7	17.3	-50	28.9	-17	37.6	+ 8	31.8	-8	30.4	-12	27.5	-20	-	-	53.5	+54
	2	436	442	+ 1	460	+ 6	470	+ 8	448	+3	447	+ 3	427	- 2	-	-	445	+ 2
	3	141	162	+15	159	+13	140	- 1	127	-10	117	-17	114	-19	-	-	139	- 1
	Mean			-11		+ 1		+ 5		-5		- 9		-14				+18
Ins. Org.	1	113	263	+133	216	+91	286	+155	310	+174	322	+184	331	+192	116	+ 3	266	+135
	2	760	758	0	753	- 1	749	- 1	799	+ 5	632	-17	-	-	810	+ 7	708	- 7
	3	46	14	-70	32	-30	14	-70	64	+39	36	-22	96	+109	208	+352	133	+189
	Mean			+21		+20		+28		+73		+48		+150		+121		+106
Sol. Org.	1	410	330	-20	329	-20	266	-35	234	-43	270	-34	255	-38	-	-	114	-72
	2	364	390	+ 7	387	+ 6	331	- 9	313	-14	425	+16	-	-	-	-	227	-38
	3	200	211	+ 6	240	+20	207	+ 4	142	-29	189	- 5	140	-30	-	-	55	-72
	Mean			- 1		+ 2		-13		-29		- 8		-34				-61

[a] Calculated by taking the difference of the two values in µg/ℓ and dividing by the initial value.

[b] Sample Nos. 1, 2, and 3 represent samples from Antioch, Rodeo, and Fort Baker, respectively.

[c] Analysis made immediately after addition of acid preservative.

[d] Percentage change calculated on the basis of the second day's NO_3-N concentration (see text).

TABLE 14

CHANGES IN CONCENTRATION (ACTUAL AND PER CENT[a]) OF NITROGEN FORMS AT 4 °C WITH 40 mg/ℓ Hg^{++} ADDED AS A PRESERVATIVE

Nitrogen Form	Sample No.[b]	Time of Storage, days														
		0	1		3		8		15		22		36		42	
		µg/ℓ	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%	µg/ℓ	%
NO_3	1	70	75	+ 7	75	+ 7	70	0	87	+24	87	+24	67	- 4	83	+19
	2	~ 0	47	-	42	-11[c]	40	-15[c]	10	-79[c]	42	-11[c]	50	+ 6[c]	55	+14[c]
	3	115	125	+ 9	130	+13	130	+13	127	+10	135	+17	127	+ 9	128	+11
	Mean			+ 8		+ 3		- 1		-15		+ 9		+ 4		+15
NO_2	1	2.98	2.88	- 3	2.80	- 6	2.21	-26	1.58	-47	1.45	-51	1.52	-49	1.94	-34
	2	6.67	7.37	+11	7.28	+ 9	6.86	+ 3	3.64	-45	3.96	-41	4.13	-38	3.97	-40
	3	6.72	7.82	+16	7.34	+ 9	6.50	- 3	4.07	-39	3.62	-46	3.82	-43	3.97	-41
	Mean			+ 9		+ 4		- 9		-44		-46		-43		-38
NH_3	1	34.7	33.3	- 4	33.3	- 4	36.2	+ 4	17.4	-50	17.4	-50	21.7	-37	52.1	+50
	2	436	465	+ 7	-	-	472	+ 8	450	+ 3	465	+ 7	475	+ 9	478	+10
	3	141	149	+ 6	148	+ 5	130	- 8	114	-19	122	-13	149	+ 6	156	+10
	Mean			+ 3		0		+ 1		-22		-19		- 7		+23
Insoluble Organic	1	113	116	+ 3	214	+89	299	+165	223	+97	125	+10	92	-19	223	+97
	2	760	706	- 7	-	-	640	-16	606	-20	636	-16	862	+13	687	-10
	3	46	62	+35	32	-30	64	+39	125	+171	3	-35	60	+30	122	+165
	Mean			+10		+30		+63		+83		-14		+ 8		+88
Soluble Organic	1	410	415	+ 1	291	-29	282	-30	336	-18	368	-10	317	-23	171	-58
	2	364	379	+ 4	-	-	388	+ 6	430	+18	386	+ 6	96	-74	266	-27
	3	200	203	+ 2	167	-16	168	-16	201	0	257	+29	112	-44	61	-70
	Mean			+ 2		-23		-13		0		+ 8		-47		-52

[a]Calculated by taking the difference of the two values in µg/ℓ and dividing by the initial value.

[b]Sample Nos. 1, 2, and 3 represent samples from Antioch, Rodeo, and Fort Baker, respectively.

[c]Percentage change calculated on the basis of the second day's NO_3-N concentration (see text).

d) Stored at 4°C; mercury preservative.

There was no consistent or significant variation of the average NO_3-N concentration from its initial value over the 42-day storage period.

2) Nitrite Nitrogen

It was not possible, by any of the methods of preservation studied, to prevent a significant decrease in the concentration of NO_2-N over the 42-day storage period.

a) Stored at 4°C; no preservative.

For a period of 3 days, no significant change took place in the NO_2-N concentration in the unpreserved sample stored at 4°C. However, after 3 days, the NO_2-N began to decrease significantly and after 42 days of storage had decreased by between 60-70 per cent of its initial value.

b) Stored at -10°C; no preservative.

Again the NO_2-N concentration remained constant for about 3 days and then decreased to a value which was 40-50 per cent below its initial value after the 42-day storage period.

c) Stored at 4°C; acid preservative.

There was an immediate decrease of NO_2-N to 25 per cent of its initial value. After the 42 days of storage, only about 15 per cent of the original NO_2-N remained. All samples from the three different areas showed a residual NO_2-N of about 0.8 $\mu g/\ell$ after 42 days of storage which seemed to be independent of the original NO_2-N concentration. It would seem to indicate that the decomposition of nitrite had proceeded to completion.

d) Stored at 4°C; mercury preservative.

The NO_2-N concentration remained essentially constant for a period of 7 days after which it began to decrease until the end of the 42-day storage period when it reached a value which was 60 per cent of its original concentration.

3) Ammonia Nitrogen

The NH_3-N concentrations in samples preserved by all of the four methods tested fluctuated erratically. Because of this fluctuation it was very difficult to detect any significant trends in the concentration of NH_3-N throughout the 42-day storage period.

a) Stored at 4°C; no preservative.

The average NH_3-N concentration fluctuated from about -30 to +120 per cent of its initial value with no significant trend discernable in the individual sample values.

b) Stored at -10°C; no preservative.

The NH_3-N values were again erratic but were mainly lower than the initial value throughout the 42-day period of storage.

c) Stored at 4°C; acid preservative.

In the acid-preserved samples the average NH_3-N values fluctuated only between $\pm$ 15 per cent of the initial concentration. No trend was observable in the NH_3-N values throughout the 42-day storage period.

d) Stored at 4°C; mercury preservative.

For a period of 10 days the average NH_3-N concentration in these samples remained close to the initial concentration. After this time, however, the NH_3-N values fluctuated erratically for the remainder of the storage period.

4) Insoluble Organic Nitrogen

In common with the ammonia nitrogen results erratic fluctuation was noted both for insoluble organic nitrogen and soluble organic nitrogen except for the acid-preserved samples where a consistent trend was noted for both of these forms of nitrogen. It should be pointed out that the scatter of points obtained for soluble and insoluble forms of organic nitrogen (especially the latter) is the result of attempting to measure small variations in concentration by the difference between two large values, which in turn were calculated from optical density values originally containing large reagent blanks. The greater fluctuation of the insoluble organic nitrogen values may be attributed to the difficulty of maintaining an homogeneous suspension of sediment material during the splitting of the original sample into sub-samples for various preservation methods.

a) Stored at 4°C; no preservative.

For the first 17 days of storage insoluble organic nitrogen concentration was between 10-15 per cent lower than the initial value. After this period the average insoluble organic nitrogen concentration fluctuated erratically but tended to be higher than its initial value.

b) Stored at -10°C; no preservative.

The freezing technique of preservation produced erratic values of average insoluble organic nitrogen during the 42-day storage period and no trend in the results could be discerned.

c) Stored at 4°C; acid preservative.

After an initial period of 3 days, when the average insoluble organic nitrogen was between 20-30 per cent of its initial value, the average values became very high and erratic.

d) Stored at 4°C; mercury preservative.

There was a steady increase of average insoluble organic nitrogen to 63 per cent of its initial value over the first 8 days of storage. After this time the average value fluctuated eratically.

5) Soluble Organic Nitrogen

a) Stored at 4°C; no preservative.

For a period of 15 days the unpreserved samples stored at 4°C showed little variation from the initial value, however, after 15 days the soluble organic nitrogen decreased to 50 per cent of its initial value.

b) Stored at -10°C; no preservative.

For a period of about 20 days the average soluble organic nitrogen in the frozen but unpreserved sample remained close to its initial value but

after this time it decreased to a value of between 30-50 per cent of its initial concentration.

c) Stored at 4°C; acid preservative.

Average soluble organic nitrogen values were generally lower than the average initial concentration using this method of preservation. Moreover they tended to progressively decrease as storage progressed.

d) Stored at 4°C; mercury preservative.

The mercury preservative held the average soluble organic nitrogen values close to its original value over about a 20-day period. After this time however, the average soluble organic nitrogen decreased to about 50 per cent of its initial value.

6) Total Nitrogen

If all the forms of nitrogen present are summed one can arrive at a total nitrogen figure for each sub-sample. These total nitrogen values for each sub-sample are summarized in Table 15 and illustrated in Figure 7 through 10. It will be noted that in all cases there seems to be a downward trend in the results as the storage period progresses, for all methods of preservation. This would indicate, if the results are statistically significant, that a loss of nitrogen occurs during the period of storage for all techniques of preservation.

To assess the statistical significance of the total nitrogen figures is not a straightforward task. The most imprecise analysis is the total unoxidized nitrogen test and unfortunately this quantity constitutes the bulk of the nitrogen present in the samples and is used for the calculation of insoluble organic nitrogen content. Inspection of Table 4 shows three values for the standard deviation and coefficient of variation of the total unoxidized nitrogen analysis from which a mean coefficient of variation can be calculated as 11.5 per cent. Since the samples used in the preservation study had a total unoxidized nitrogen content in the same general range (about 0.4-1.56 mg N/ℓ) to the samples used for determining the precision of the test (about 0.3-0.7 mg N/ℓ) a mean of the standard deviations for the total unoxidized nitrogen test obtained in the precision study was applied to the samples used in the preservation study. A similar procedure was adopted for the total soluble unoxidized nitrogen test.

TABLE 15

CHANGES IN TOTAL NITROGEN CONTENT OF SAMPLES DURING DIFFERENT PRESERVATION TECHNIQUES

Preservative	Sample No.	Total Nitrogen, mg/ℓ							
		Time of Storage, days							
		0	1	3	8	15	22	36	42
4°C None	1	0.63	0.65	0.59	0.57	0.58	0.56	--	0.53
	2	1.57	1.63	1.58	1.59	1.61	1.69	--	1.52
	3	0.51	0.58	0.51	0.47	0.54	0.62	--	0.48
− 10°C None	1	0.63	0.68	0.61	0.61	0.62	--	--	0.51
	2	1.57	1.54	1.50	1.62	1.43	1.34	--	1.46
	3	0.51	0.47	0.57	--	--	--	0.46	0.53
4°C Acid	1	0.63	0.67	0.68	0.69	0.72	0.71	--	0.53
	2	1.57	1.71	1.63	1.62	1.59	--	--	1.49
	3	0.51	0.51	0.57	0.50	0.46	0.47	0.49	0.47
4°C Mercury	1	0.63	0.64	0.54	0.69	0.66	0.60	0.50	0.53
	2	1.57	1.60	--	1.55	1.50	1.53	1.49	1.49
	3	0.51	0.55	0.48	0.50	0.57	0.52	0.45	0.47

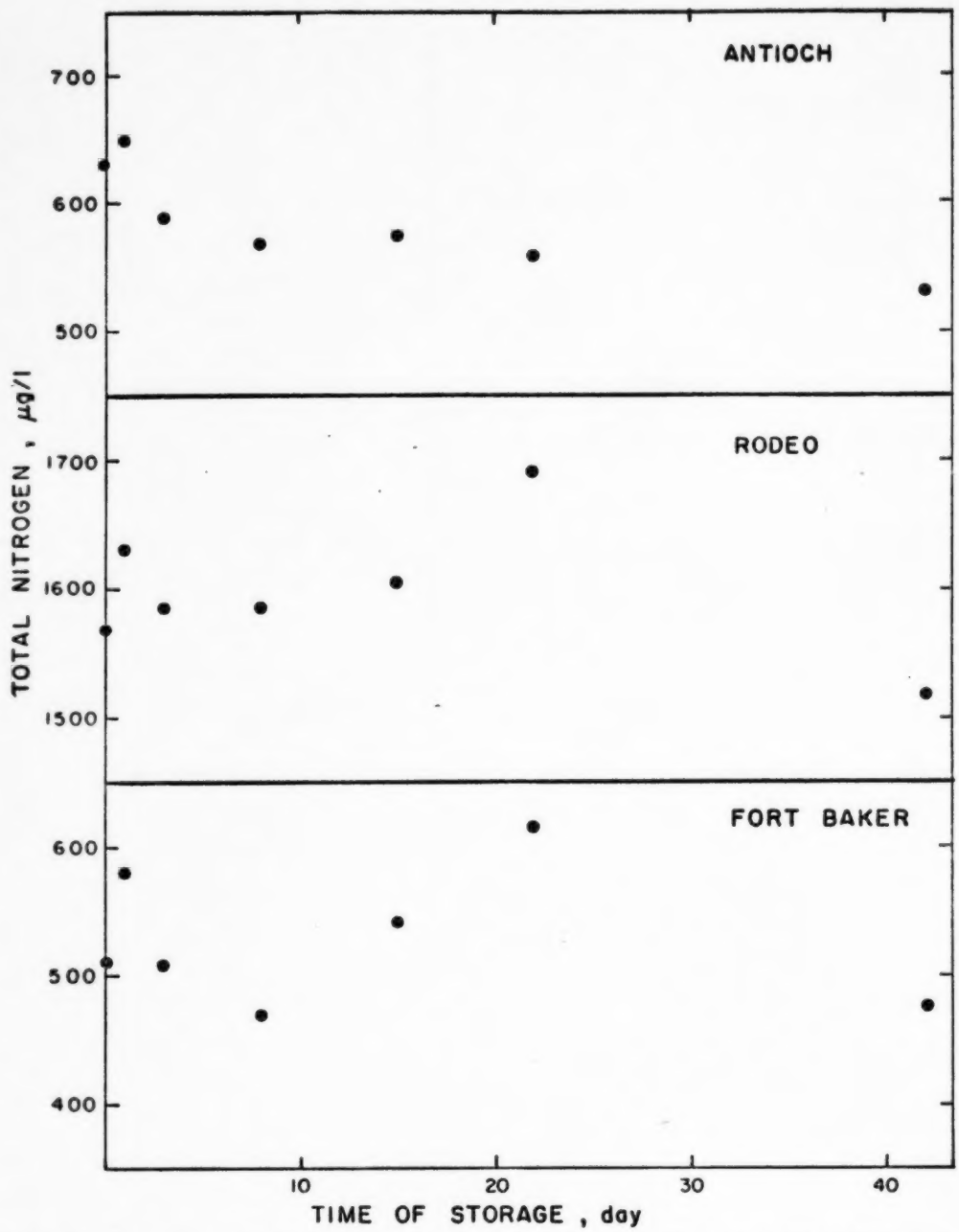

FIGURE 7 . VARIATION OF TOTAL NITROGEN CONCENTRATION DURING STORAGE AT 4°C WITH NO PRESERVATIVE

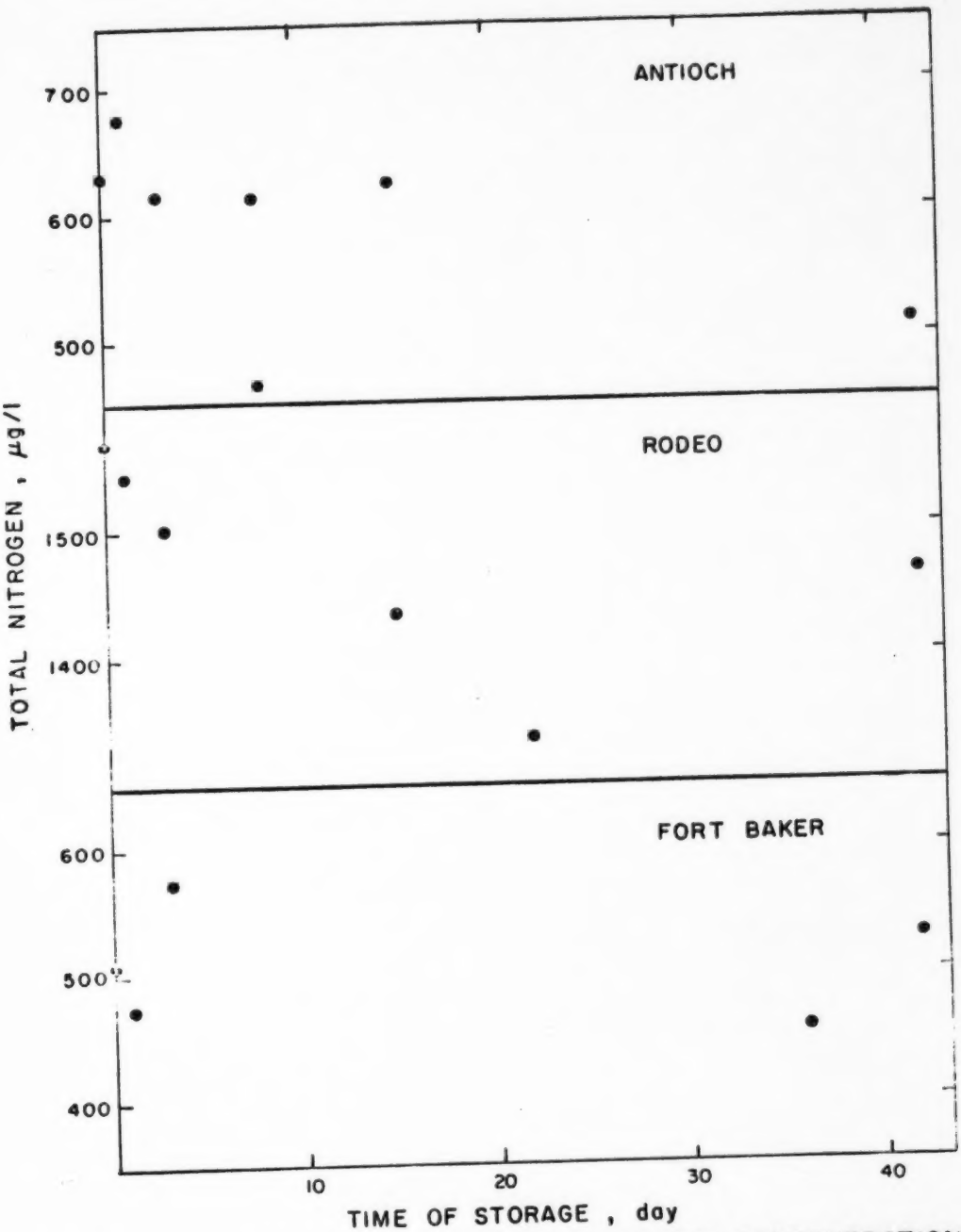

FIGURE 8 . VARIATION OF TOTAL NITROGEN CONCENTRATION DURING STORAGE AT −10°C WITH NO PRESERVATIVE

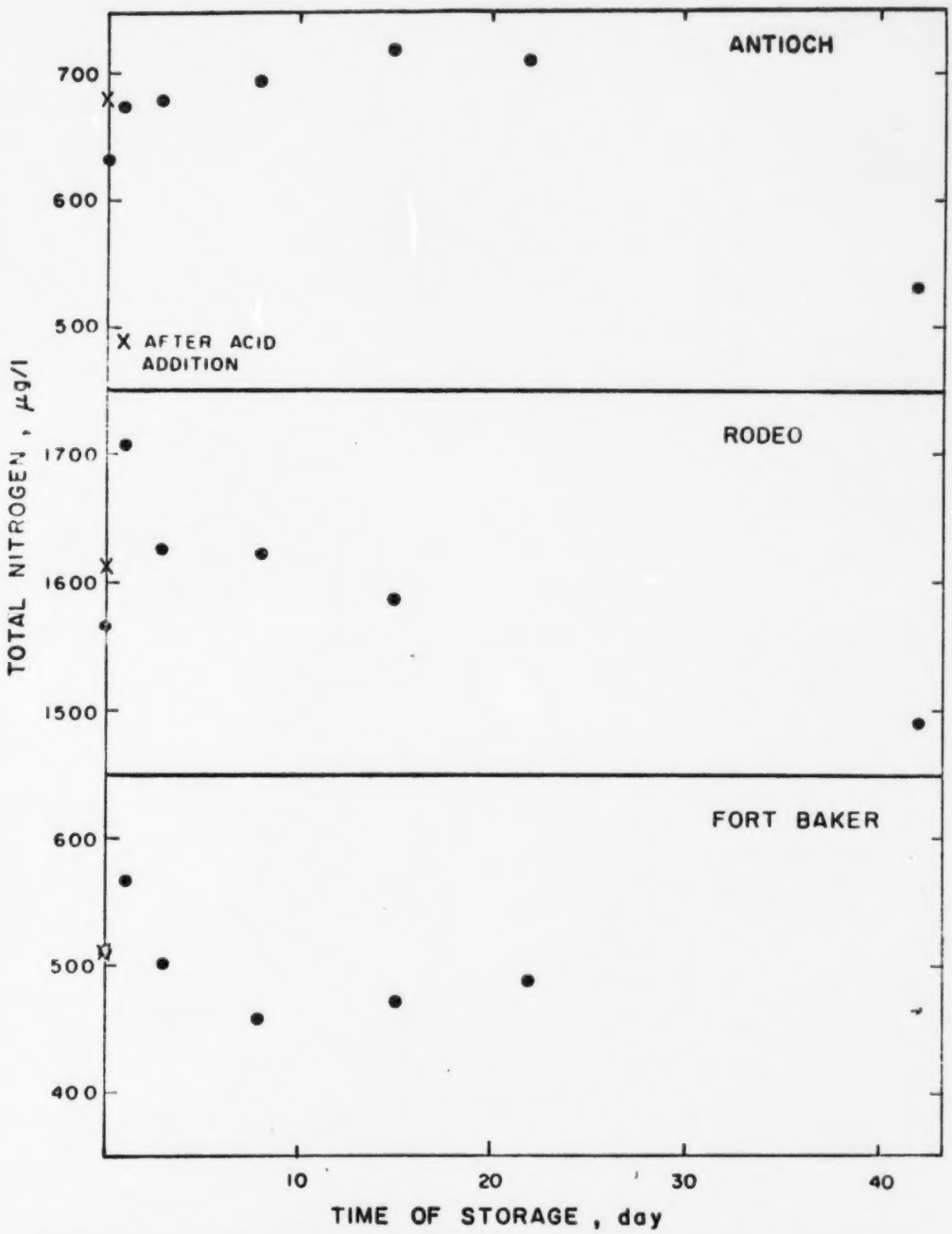

FIGURE 9 . VARIATION OF TOTAL NITROGEN CONCENTRATION DURING STORAGE AT 4°C WITH 2ml 5% H_2SO_4/l ADDED AS A PRESERVATIVE

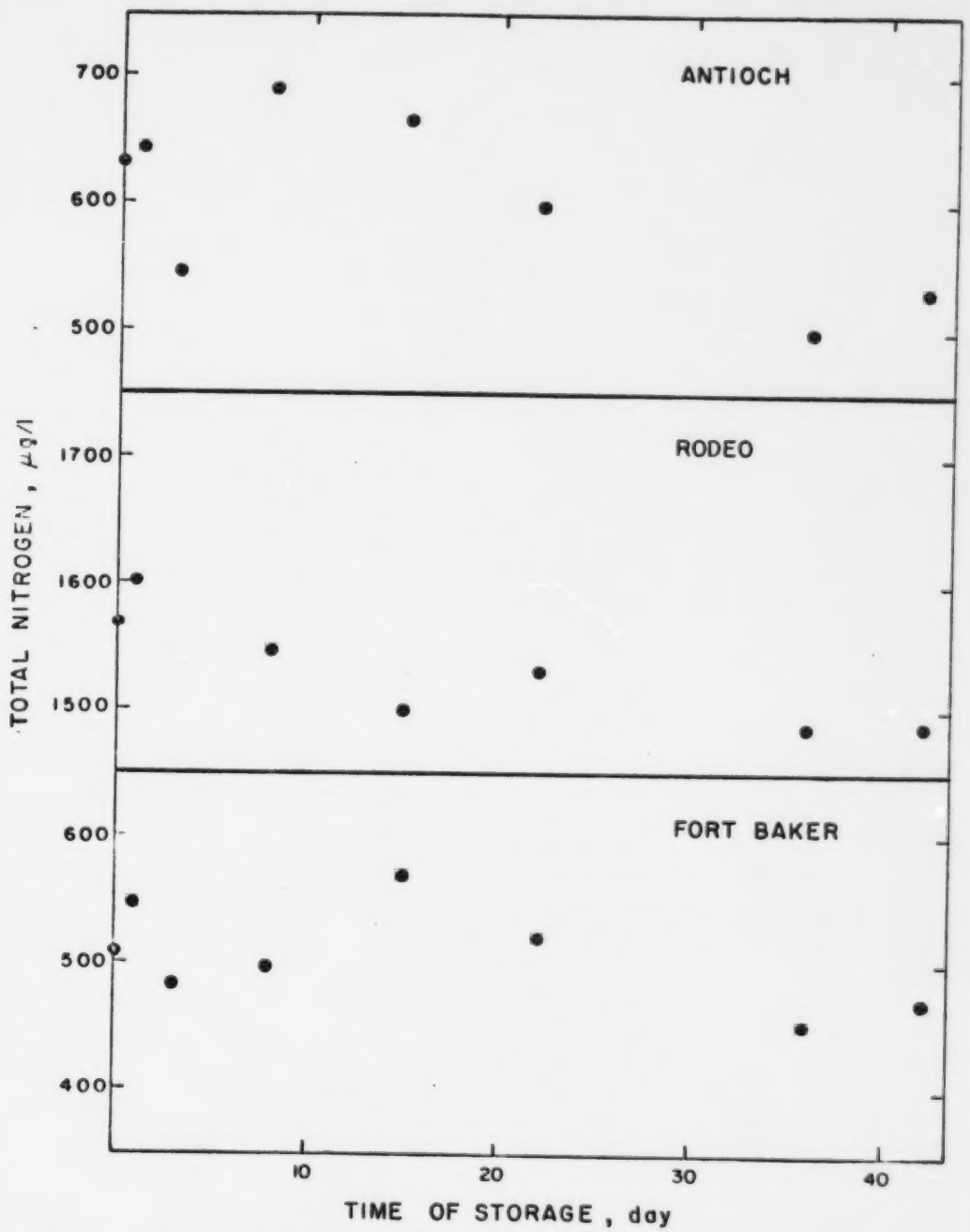

FIGURE 10 . VARIATION OF TOTAL NITROGEN CONCENTRATION DURING STORAGE AT 4°C WITH 40 mg/l Hg^{++} ADDED AS A PRESERVATIVE

In computing the standard deviation of the total nitrogen results, the error contribution of the two aforementioned analyses is major. The error from the analyses of NO_3-N, NO_2-N, and NH_3-N is very small. Thus summarizing the standard deviations (σ) of the various analyses we have:

Analysis	σ
NO_3-N	5 µg/ℓ
NO_2-N	0.1 µg/ℓ
NH_3-N	3.7 µg/ℓ
Total Unoxidized Nitrogen	51.5 µg/ℓ
Soluble Unoxidized Nitrogen	12.3 µg/ℓ

For the insoluble organic nitrogen

$$\sigma = \sqrt{(51.5)^2 + (12.3)^2} = \sqrt{2801} \simeq 53 \text{ µg/ℓ} .$$

For the soluble organic nitrogen

$$\sigma = \sqrt{(12.3)^2 + (3.7)^2} = \sqrt{165} \simeq 12.8 \text{ µg/ℓ} .$$

For the total nitrogen

$$\sigma_{\text{Total N}} = \sqrt{(53)^2 + (12.8)^2 + (3.7)^2 + (5)^2 + (0.1)^2}$$

$$= \sqrt{3005} \simeq \underline{\underline{55 \text{ µg/ℓ}}} .$$

For significance at the 95 per cent confidence level results should differ by greater than 2 σ.

Thus results should differ by

$$2 \times \sigma_{\text{Total N}} \simeq 2 \times 55 \simeq \underline{\underline{110 \text{ µg/ℓ}}}.$$

It should be stated that this is a very crude estimate of accuracy and significance, yet it is believed that the estimate is conservative. With this fact in mind, inspection of Figures 7 through 10 reveals that only isolated points would lie outside the limits of significance. It can therefore be stated that doubt exists as to whether there is a real decrease in total nitrogen content during storage with the different preservative techniques for a period of 42 days.

VI. CONCLUSIONS AND RECOMMENDATIONS

1. Analytical Techniques

Techniques suitable for the analysis of NO_3, NO_2, NH_3, and soluble and insoluble organic nitrogen in estuarine waters have been developed and tested for applicability on samples from three areas of San Francisco Bay typical of the ocean, estuarine, and fresh water environment.

The recovery and precision of the various analytical techniques at levels commonly encountered in San Francisco Bay waters have been determined and presented in this report. All techniques except the total unoxidized nitrogen analysis showed satisfactory precision at the levels of interest. It is suggested that inhomogeneity of the samples may be the cause of the lack of precision in the total unoxidized nitrogen test since the variation was always caused by erratically high results.

2. Choice of Preservative

Four preservation techniques were investigated for their ability to conserve the initial concentrations of the various forms of nitrogen.

Table 16 summarizes the suitability of the four preservation methods for stabilizing the concentrations of various forms of nitrogen. From a review of Table 16 it can be seen that the best all-round preservative seems to be the use of 40 mg/ℓ Hg^{++} with storage at 4°C. In all instances except for the organic nitrogen forms (where analytical variation makes the drawing of firm conclusions a difficult task) it seems to be possible to store the samples for a period of about 8 days before significant changes in the concentration of the various forms of nitrogen take place.

It is definitely recommended that acid preservation of the samples, by adding 2 mℓ of 5% H_2SO_4/ℓ of sample, not be used as a preservative technique. The only forms of nitrogen which can be preserved successfully by acid addition seem to be nitrate and possibly ammonia nitrogen. As might be expected from the following reaction attributed to Van Slyke

$$HNO_2 + R\text{-}NH_2 \xrightarrow{H^+} N_2 \uparrow + ROH + H_2O,$$

the addition of acid causes an immediate decrease in the concentration of NO_2-N. The use of a mercury preservative in conjunction with deep-freezing may be an even better preserving technique than the use of mercury in a sample stored at 4°C. In view of these conclusions and this supposition it is recommended that the Preservation Study to be conducted for the various forms of phosphorus in San Francisco Bay waters will omit the use of

TABLE 16

SUITABILITY OF THE VARIOUS PRESERVATION METHODS FOR VARIOUS PERIODS OF STORAGE[a]

Nitrogen Form	Storage Temperature															
	4°C				4°C				4°C				Deep Freeze			
	Type of Preservative															
	None				H_2SO_4				Hg^{++}				None			
	Time of Storage, days															
	3	8	15	30	3	8	15	30	3	8	15	30	3	8	15	30
NO_3-N	+	?			+	+	+	-	+	+	+	+	+	+	?	
NO_2-N	+	-			-				+	+	-		+	-		
NH_3-N	-				+	+	?		+	+	-		-			
Insoluble Organic N	+	+	+		-				?	-			-			
Soluble Organic N	+	+	+	-	+	?			?	-	-		+	+	-	

[a] + indicates suitablility; ? indicates questionable suitability; - indicates unsuitability.

sulfuric acid as a preservation technique. In place of the acid-preservation technique it is recommended that the use of mercury in conjunction with deep freezing be explored as a preservation technique for the various forms of phosphorus.

3. Experimental Procedures

The erratic nature of some of the analytical results presented in this study can be attributed to the hurried manner in which the analyses had to be carried out because of the large analytical load. Compounding this problem was the fact that there was not sufficient volume of preserved sample to carry out repeat analyses even if the time had been available. It is therefore suggested that in future studies a single water sample from the San Francisco Bay be studied by four preservation techniques rather than studying the four preservation techniques on three samples from different areas of the Bay. Using this experimental design it should be possible to obtain far more consistent values for the various forms of material being analyzed and also to have sufficient material available for repeat analyses should erratic results be obtained.

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APPENDIX I

RECOMMENDED ANALYTICAL PROCEDURES

A. AMMONIA NITROGEN

1. Apparatus

1.1. Kjeldahl flask, 500 mℓ.

1.2. Kjeldahl distillation apparatus (Precision Scientific Co. or equivalent).

1.3. Beckman DU Spectrophotometer with attachment for 10 cm cells.

2. Reagents

2.1. Demineralized distilled water. Pass distilled water over a suitable cation exchange resin (in the hydrogen form). Use for preparation of all reagents as well as in the analysis.

2.2. Phenol phthalein indicator solution: 0.5% in 50% ethyl alcohol.

2.3. Sodium carbonate solution: 2.5 per cent solution of reagent grade Na_2CO_3 in demineralized water.

2.4. Nessler reagent: Use reagent grade chemicals. Dissolve 70 g KI in approximately 200 mℓ of demineralized water; add 100 g HgI_2. To this solution add slowly with stirring a cooled solution of 160 g solid NaOH in 500 mℓ demineralized water (for convenience, 210 mℓ of a 50% NaOH solution which is commercially available may be substituted for 160 g solid NaOH). Dilute to 1 ℓ. Set aside for 1-2 days and decant the clear supernatant. Store in a dark bottle.

2.5. Stock standard ammonium chloride solution: Hellige standard solution may be used but should be purchased in small lots so that after one use the remainder of the bottle may be discarded; or dissolve 3.819 g anhydrous NH_4Cl in demineralized water and dilute to 1 ℓ.

$$1\ m\ell \equiv 1\ mg\ NH_3\text{-}N$$

2.6. Standard ammonium chloride solution: Dilute 10 mℓ stock standard ammonium chloride solution to 1 ℓ with demineralized distilled water.

$$1\ m\ell \equiv 10\ \mu g\ NH_3\text{-}N$$

2.7. Boileezers: Fisher Scientific Co.

2.8. Boric acid solution: 2 per cent in demineralized distilled water.

3. Procedure

3.1. Before starting a set of analyses, steam out the distillation apparatus for about 1 hour. Rinse all glassware with distilled water.

3.2. Measure a 200-mℓ sample into a 500-mℓ Kjeldahl flask. Add 4-5 boileezers and dilute to approximately 350 mℓ with demineralized distilled water. Add 3-4 drops of phenolphthalein indicator solution followed by Na_2CO_3 solution sufficient to produce a pink color (usually 2-3 drops). Immediately connect the flask to the distillation system. Bring to a boil, being careful to avoid foaming.

3.3. Collect about 200 mℓ distillate in 20 mℓ of 2% boric acid solution in a 300-mℓ Erlenmeyer flask. Begin the distillation with the delivery tube immersed in the boric acid solution. Toward the end of the distillation place the flask upright and allow the last 50-75 mℓ of distillate to drain down the tube.

3.4. Make up the volume of the distillate to 250 mℓ with demineralized distilled water. Add 10 mℓ Nessler reagent. Mix well and allow to stand 15 minutes. Measure the optical density against distilled water at 467 mμ with a Beckman DU Spectrophotometer using a 10-cm cell. When a series of samples are being handled, allow one minute between the addition of Nessler reagent to each sample in order to allow time for manipulation prior to reading the optical density of the solution.

3.5. Carry a reagent blank through the complete procedure and subtract this value from the optical density of the samples.

3.6. Preparation of standard curve. Dilute 20 mℓ standard NH_4Cl solution to 200 mℓ. Measure 0, 1, 2, 5, 10, 20, 40, and 60 mℓ portions of this solution into Erlenmeyer flasks and add sufficient demineralized distilled water to make up to a total volume of 100 mℓ. Add 4 mℓ Nessler reagent to each standard; mix; and after 15 minutes, measure the optical density at 467 mμ. After subtracting the blank plot a graph relating optical density to μg NH_3-N.

4. Calculation

4.1. If the slope of the best straight line from the standard curve is used

$$\mu g\ NH_3\text{-}N/\ell = \frac{\text{OD Sample (OD Units)}}{\text{Sample Vol. (mℓ)}} \times \frac{\text{Final Vol. Nesslerized (mℓ)}}{\text{Vol. Used for Std. Curve (mℓ)}} \times \frac{1}{\text{Slope Std. Curve } \left(\frac{\text{OD Units}}{\mu g}\right)} \times \frac{1000}{\frac{\text{(mℓ)}}{\text{(ℓ)}}}$$

4.2. If μg NH_3-N are read directly from the standard curve

$$\mu g\ NH_3\text{-}N/\ell = \frac{\text{Vol. Nesslerized } (m\ell)}{\text{Vol. Used for Std. Curve } (m\ell)} \times \frac{\mu g\ NH_3\text{-}N \text{ from Std. Curve}}{\text{Sample Vol. } (m\ell)} \times \frac{1000\ (m\ell)}{1\ (\ell)} .$$

5. Precision and Accuracy

5.1. Using the recommended procedure, the limit of detectability is about 10 μg NH_3-N/ℓ. Beer's law is reasonably well obeyed over a wide range. If necessary, the limit of detectability can be reduced to about 3 μg NH_3-N/ℓ by distilling a 500-mℓ sample in an 800-mℓ Kjeldahl flask and diluting the distillate to 200 mℓ.

5.2. The recovery of added NH_3-N from Bay water samples of salinity varying from that of fresh water to that of sea water is 95 per cent.

5.3. The precision of the recommended technique is

C_v = 1.8 per cent at about 100 μg NH_3-N/ℓ;

C_v = 6.9 per cent at about 60 μg NH_3-N/ℓ; and

C_v = 13.0 per cent at about 35 μg NH_3-N/ℓ.

B. TOTAL UNOXIDIZED NITROGEN (WHOLE SAMPLE)

1. Apparatus

1.1. See NH_3-N method.

2. Reagents

2.1. Demineralized distilled water: See NH_3-N method.

2.2. Hengar selenized granules.

2.3. Sulfuric acid: reagent grade, concentrated.

2.4. Kelpak bags; No. 2P; Harshaw Scientific Co.; each packet contains 10 g K_2SO_4, 0.30 g $CuSO_4$, and 0.1 g Pumice.

2.5. Sodium hydroxide solution, 50%; Fisher reagent.

2.6. Boric acid solution: See NH_3-N method.

2.7. Nessler reagent: See NH_3-N method.

3. Procedure

3.1. Measure a 200-mℓ sample into a 500-mℓ Kjeldahl flask. Add 2 or 3 Hengar granules, one bag of Kelpak and 20 mℓ conc. H_2SO_4. Place the flask in the digestion unit and boil the contents on high heat until the first fumes of SO_2 appear. Turn heat to low and carry the digestion out for a further 40 min.

3.2. Switch off the heater and cool the solution. Remove the flask and stopper with a No. 6 rubber stopper. Dilute the solution with about 300 mℓ demineralized distilled water and mix thoroughly. (Heat of mixing is generated in this step.) When the bottom of the flask has cooled so that it is just warm to the touch, introduce 50 mℓ of 50% NaOH and immediately place the flask on the distillation apparatus which has been previously set up with a receiving flask containing 20 mℓ 2% boric acid solution.

3.3. The remainder of the procedure is as described for NH_3-N. Carry a blank through the entire process and deduct its optical density from the sample values.

4. Calculation

4.1. Exactly the same calculations apply here as for the NH_3-N determinations. However, the result is now expressed as µg total unoxidized N/ℓ.

5. Precision and Accuracy

5.1. The recovery of added NH_3-N by the total unoxidized nitrogen technique from Bay water samples of salinity varying from that of fresh water to that of sea water is about 98 per cent.

5.2. The precision of the recommended technique is dependent on the homogeneity of the samples which is greatly influenced by their suspended matter content. Coefficients of variation of between 3.6 and 18.2 per cent were obtained.

C. SOLUBLE UNOXIDIZED NITROGEN

1. Apparatus

1.1. See total unoxidized nitrogen. In addition to this apparatus a Millipore filtration unit and washed HA Millipore filters are required. Soak the Millipore filters in demineralized distilled water; replace the water after 2 hours and soak the filters overnight.

2. Reagents

2.1. See total unoxidized nitrogen.

3. Procedure

3.1. Filter the sample through an HA Millipore filter and then proceed as for total unoxidized nitrogen, carrying out the analyses on the filtrate.

4. Calculation

4.1. See total unoxidized nitrogen.

5. Precision and Accuracy

5.1. A coefficient of variation of 3.6 per cent was obtained for replicate samples with a mean soluble unoxidized nitrogen content of 340 µg/ℓ.

D. NITRITE NITROGEN

1. Apparatus

1.1. Millipore Filtration Apparatus.

1.2. Millipore Filters, Type HA.

1.3. Volumetric Flasks, 100-mℓ capacity.

1.4. Spectrophotometer: Beckman DU or equivalent, 10 cm cells.

2. Reagents

2.1. Sulfanilic acid solution: Dissolve 27.2 g $KHSO_4$ and 3.46 g sulfanilic acid in distilled water and dilute to 1 ℓ.

2.2. N-(1-Naphthyl)-ethylenediamine dihydrochloride solution: Dissolve 0.4 g N-(1-Naphthyl)-ethylenediamine dihydrochloride in distilled water and dilute to 1 ℓ. Store in a dark glass bottle and discard when a significant reagent blank begins to appear in the test.

2.3. Stock standard sodium nitrite solution: Dissolve 0.2463 g anhydrous reagent grade $NaNO_2$ in distilled water and make up to 1 ℓ. Preserve by adding 1 mℓ chloroform.

$$1\ m\ell \equiv 50\ \mu g\ NO_2\text{-}N$$

2.4. Standard sodium nitrite solution: Dilute 10.0 mℓ stock standard sodium nitrite solution to 1 ℓ. Preserve by adding 1 mℓ chloroform. Prepare fresh each week.

$$1\ m\ell \equiv 0.5\ \mu g\ NO_2\text{-}N$$

3. Procedure

3.1. Clarify the sample by filtering through an HA Millipore filter.

3.2. Measure a suitable volume of filtrate (not more than 60 mℓ) into a 100-mℓ volumetric flask. Adjust the temperature of the sample to between 15-25°C.

3.3. Dilute the sample to at least 20 mℓ (if necessary) and add 5.0 mℓ of sulfanilic acid solution. Mix and allow to react for between 2-8 minutes. Add 5.0 mℓ N-(1-naphthyl)-ethylenediamine dihydrochloride solution. Mix and set aside for at least 10 minutes.

3.4. Make up to 100 mℓ with distilled water and measure the optical density of the solution at 550 mμ against a reagent blank in a spectrophotometer with a 10-cm light path.

3.5. Because of the linear nature of the standard curve, a single or duplicate standard is adequate to establish the slope of the curve.

4. Calculation

4.1. $\mu g\ NO_2\text{-}N/\ell = \dfrac{\text{optical density of sample} \times 1000}{\text{slope of standard curve} \times \text{sample vol. } (m\ell)}$.

4.2. Slope of standard curve = $\dfrac{\text{optical density of standard}}{\text{nitrite content of standard } \mu g\ NO_2\text{-}N}$.

5. Precision and Accuracy

5.1. The coefficient of variation of the NO_2-N method recommended herein was found to be never greater than 2.6 per cent.

E. NITRATE NITROGEN

1. Apparatus

1.1. Spectrophotometer: Bausch and Lomb Spectronic 20 with adapter for 1-inch cells or equivalent.

1.2. Wire racks. BKH Catalog No. 60935 are suitable.

1.3. Stirred water bath. A 12-inch x 18-inch Lindberg Hot Plate with a metal pan and electric stirrer has been found to be quite satisfactory.

2. Reagents

2.1. Stock standard nitrate solution, 100 mg/ℓ. Dissolve 0.7218 g KNO_3 in distilled water and make up to 1 ℓ.

2.2. Standard nitrate solution, 10 mg/ℓ. Dilute 10 mℓ stock standard nitrate solution to 100 mℓ. Prepare immediately before using.

2.3. Sodium chloride solution: 30 per cent aqueous solution.

2.4. Sulfuric acid solution: Add 500 mℓ conc. H_2SO_4 to 125 mℓ distilled water. Cool before using and keep tightly stoppered to prevent adsorption of moisture.

2.5. Brucine–sulfanilic acid solution: Dissolve 1 g of brucine sulfate and 0.1 g of sulfanilic acid in 70 mℓ of hot distilled water. Add 3 mℓ of conc. HCl, cool and make up to 100 mℓ. This solution is stable for many months.

3. Procedure

3.1. Set up the required number of 1-inch colorimeter tubes in a suitable rack, spacing them in alternate slots. Include a tube for the reagent blank and as many standards as desired.

3.2. To each tube add 10 mℓ of sample (or an aliquot diluted to 10 mℓ). Set up a reagent blank and standards.

3.3. Place the rack in a cool water bath and add 2 mℓ of NaCl solution. Mix and add 10 mℓ H_2SO_4 solution. Mix again thoroughly* by swirling and allow to cool. At this point, if any turbidity or color is present or if the tubes have not been matched, they should be dried off and a "sample blank" value read against the reagent blank tube at 410 mμ.

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*Although thorough mixing is essential this should be accomplished by swirling by hand. In no case should a Vortex mixer be used.

3.4. Replace the rack of tubes in the cool water bath and add 0.5 mℓ brucine–sulfanilic acid solution. Swirl the tubes to mix and place the rack of tubes in a well-stirred water bath at a temperature of not less than 95°C.

3.5. After 20 min, remove the samples and immerse them in a cold water bath. When thermal equilibrium is reached (at approximately room temperature), dry off the tubes with tissue and read the standards and samples against the reagent blank at 410 mμ in the spectrophotometer.

4. Calculation

4.1. To obtain a standard curve, subtract the "sample blanks" from the final optical density readings and plot the resultant optical densities against mg NO_3-N/ℓ. Resultant optical density readings for the samples are obtained by subtracting their "sample blank" values from their final optical density values. Concentrations of NO_3-N may be determined by either of the following methods.

4.2. If a large number of samples is involved, an equation may be obtained from the standard curve for rapid calculation of results. Alternately, the mg NO_3-N/ℓ may be read directly from the standard curve.